IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cantor *et al.*

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For: *USE OF NUCLEOTIDE ANALOGS IN THE ANALYSIS OF OLIGONUCLEOTIDE MIXTURES AND IN HIGHLY MULTIPLEXED NUCLEIC ACID SEQUENCING*

Art Unit: 1634

Examiner: Chakrabarti, Arun K.

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AMENDMENT

Commissioner for Patents
U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

Responsive to the Office Action mailed April 3, 2002, consideration and entry of the following amendment and consideration of the following Remarks are respectfully requested.

IN THE CLAIMS:

Please replace claims 4, 6, 17, 27, 28, 31, 33, 36 and 42 with amended claims 4, 6, 17, 27, 28, 31, 33, 36 and 42 as follows:

4. (Amended) The method of claim 1, wherein a mass-matched deoxynucleotide is deoxyinosine, 5-nitroindole, 3-nitropyrrole , 3-methyl 7-propynyl isocarbostyril, 5-methyl isocarbostyril or 3-methyl isocarbostyril.

6. (Amended) The method of claim 5 that is a method for determining nucleotide sequences of a plurality of target nucleic acid molecules, comprising:

synthesizing extension products of the target nucleic acid in the presence of chain terminating nucleotides and mass-matched nucleotides;

determining the mass of each extension product; and

calculating a mass shift from a period for the mass of each extension

product,

C2 whereby the nucleotide sequences of the target nucleic acids are determined by determining the nucleotide that corresponds to each mass shift.

17. (Amended twice) A method for detecting one or a plurality of target nucleic acid(s) molecules or one or a plurality of nucleotides therein, comprising:

(a) copying the target nucleic acid molecule(s) in the presence of a pair-matched set of nucleotides;

(b) denaturing the resulting copies of the target(s) to produce single-stranded templates;

(c) annealing and ligating one or a plurality of partially duplex hairpin

C3 primers to the single-stranded template(s);

(d) extending the primer(s) in the presence of chain terminating nucleotides and pair-matched nucleotides to produce extension products, wherein the extension products follow a periodic mass distribution that is determined by the mass of the pair-matched nucleotide set; and

(e) detecting each of the targets or nucleotides therein by virtue of the mass shift of each extension product from its corresponding periodic reference mass.

27. (Amended) A method for detecting a plurality of target nucleic acid molecules in a sample containing nucleic acid molecules, comprising:

C4 preparing a composition containing a plurality of pair-matched nucleic acid molecules or mass-matched nucleic acid molecules from a sample comprising the target nucleic acid molecules;

analyzing the resulting composition by mass spectrometry; and

detecting target nucleic acid molecules.

28. (Amended) A process for detecting a mutation in a target nucleic acid sequence in a target nucleic acid molecule, in a sample, comprising:

a) hybridizing a primer to nucleic acid molecules in the sample, thereby producing a hybridized primer and a molecule from the sample,

wherein:

the nucleic molecules from the sample are optionally immobilized and

the primer is complementary to a sequence in the target nucleic acid sequence that is adjacent to the region suspected of containing a mutation sequence;

b) contacting the hybridized primer with a composition comprising mass-matched deoxyribonucleoside triphosphates and a chain terminating nucleotide selected from a dideoxyribonucleoside triphosphate or a 3'-deoxynucleoside triphosphate and optionally one or more deoxyribonucleoside triphosphates, such that the hybridized primer is extended until a chain terminating nucleotide is incorporated, thereby producing an extended primer; and

c) determining the mass of the extended primer, thereby determining whether a mutation is present in the target nucleic acid sequence.

31. (Amended) A process for detecting mutations in a plurality of target nucleic acid sequences in a sample, comprising:

a) hybridizing a plurality of primers to nucleic acid molecules in the sample, thereby producing hybridized primers, wherein:

the nucleic acid molecules from the sample are optionally immobilized and

each primer is complementary to a sequence of a target nucleic acid sequence that is adjacent to a region suspected of containing a mutation sequence;

b) contacting the hybridized primers with a composition comprising a chain terminating nucleotide selected from a mass-matched dideoxyribonucleoside triphosphate or a 3'-deoxynucleoside triphosphate and one or more deoxyribonucleoside triphosphates, such that the hybridized primers are extended until a chain terminating nucleotide is incorporated, thereby producing an extended primer; and

c) determining the mass of the extended primers, thereby determining whether mutations are present in the target nucleic acid sequences.

C5

33. (Amended) The method of claim 31, wherein the masses of the extended primers are determined by mass spectrometry.

C6

36. (Amended twice) A method for detecting a plurality of target nucleic acid sequences, comprising the steps of:

a) hybridizing a primer or plurality thereof to nucleic acid molecules comprising target nucleic acid sequences, wherein the primers can be extended in a 3′ direction towards the target nucleic acid sequence, and wherein the 5′ end of the hybridized mass-matched nucleic acid molecules can be selectively cleaved from the extension product;

C7

b) extending the primers in the presence of mass matched deoxyribonucleotides and a polymerase to produce extension products;

c) selectively cleaving the 5′ end of the primers from the extension products to produce portions of the primers and cleaved extension products; and

d) detecting the cleaved extension products.

42. (Amended) A method for detecting a target nucleic acid sequence, comprising:

a) hybridizing first and second primers to a nucleic acid molecule containing the target nucleic acid sequence, wherein a primer contains a selectively cleavable site at its 3′ end;

C8

b) extending the primers in the presence of mass-matched nucleotides;

c) cleaving the resulting product at the selectively cleavable sites; and

d) analyzing the masses of the cleavage products, whereby the target sequence is detected.

REMARKS

A check for the fee for a three-month extension of time accompanies this response. Any fees that may be due in connection with this paper or this application may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is needed, this paper is to be considered such Petition.

Claims 1-8, 10-20, and 25-45 are pending in this application. Claims 4, 6, 17, 27, 28, 31, 33, 36 and 42 are amended to correct minor grammatical and typographical errors. No new matter is added.

A marked up copy of the amended claims per 37 C.F.R. §1.121 is attached to this response.

THE REJECTION OF CLAIMS 4 and 8 UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 4 and 8 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicants regard as the invention. The Examiner alleges improper use of Markush language. Applicant respectfully disagrees.

Relevant Law

35 U.S.C. §112, second paragraph requires only reasonable precision in delineating the bounds of the claimed invention. The claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject matter permits. Shatterproof Glass Corp.v. Libby-Owens Ford Col, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir), cert dismissed, 106 S. Ct. 340 (1985).

The amount of detail required to be included in the claims depends on the particular invention and the prior art and is not to be viewed in the abstract, but in conjunction with whether the specification is in compliance with the first paragraph of 35 U.S.C. §112. If the claims, read in light of the specification, reasonably apprise those skilled in the art of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more:

> [i]t is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter (Bendix Corp. v United States, 600 F.2d 1364, 1369, 220 Ct. Cl. 507,514, 204 USPQ 617, 621 (1979); See, also, Carl Zeiss Stiftung v. Renishaw plc, 20 USPQ2d 1094, 1101).

Analysis

The Examiner recommends that recitation of "*wherein* a mass-matched deoxynucleotide is deoxyinosine, 5-nitroindole, 3-nitropyrrole, 3-methyl 7-propynyl isocarbostyril, 5-methyl iscarbostyril *or* 3-methyl iscarbostyril" be replaced by "*selected from the group consisting of* deoxyinosine, 5-nitroindole, 3-nitropyrrole, 3-methyl 7-propynyl isocarbostyril, 5-methyl iscarbostyril *and* 3-methyl iscarbostyril". (emphases added)

Applicant respectfully submits that the use of alternative language such as "or" in Markush groups has been held to fall within section 112, second paragraph. *In re Gaubert*, 524 F.2d 1222, 187 USPQ 664 (CCPA 1975). Exemplary acceptable alternative language includes "*wherein* R is A, B, C, *or* D." MPEP § 2173.05(h), 2100-202. (emphasis added)

"Alternative expressions are permitted if they present no uncertainty or ambiguity with respect to the question of scope or clarity of the claims." [*see* MPEP 2173.05(h), *see also*, In re Wolfrum 486 F.2d. 588, 179 USPQ 620 (CCPA 1971), which held that Section 112 cannot serve as the basis for rejecting a single claim on the ground that it embodies more than one invention (i.e., alternative)]. A Markush group is just one type of alternative form of expression. The alternatives set forth in the instant claims are not intended to be a Markush group. MPEP 2173.01 states:

> A fundamental principle contained in 35 U.S.C. 112, second paragraph is that applicants are their own lexicographers. They can define in the claims what they regard as their invention essentially in whatever terms they choose so long as the terms are not used in ways that are contrary to accepted meanings in the art. Applicant may use functional language, alternative expressions, negative limitations, or any style of expression or format of claim which makes clear the boundaries of the subject matter for which protection is sought. As noted by the Court in In re Swinehart,

439 F.2d 210. 160 USPQ 226 (CCPA 1971), a claim may not be rejected solely because of the type of language used to define the subject matter for which patent protection is sought.

Thus, a claim reciting alternative expressions is acceptable if it is unambiguous. In this instance, there is no ambiguity. The claims are not Markush claims, but rather recite each subunit as an alternative. Therefore, they are not in violation of 35 U.S.C. § 112, second paragraph.

THE REJECTION OF CLAIMS. 1-3, 5-7, 10-12, AND 25-27 UNDER 35 U.S.C. § 102(b)

Claims 1-3, 5-7, 10-12, and 25-27 are rejected as allegedly being anticipated by Brennan (U.S. Patent 5,174,962). The Examiner alleges that Brennan discloses a method for identifying nucleotides at one or more base positions and determining the nucleotide sequence of a plurality of target nucleic acid molecules in which extension products of the target nucleic acid molecules are synthesized using either mass-matched (Abstract; Column 5, line 25 to Column 6, line 8; Examples 1-4; and Schemes D and E) or pair-matched (Abstract; Column 6, line 65 to Column 10, line 64) nucleotides and chain terminating nucleotides, calculating a mass shift from a period for the mass of each extension product (Abstract; Column 5, line 25 to Column 6, line 8; Examples 1-4; and Figure 2B), and identifying the nucleotides at each base position that correspond to each mass shift (Column 6, lines 10-64). It is further alleged that Brennan discloses a method in which the mass-matched deoxynucleotides are identical (Figures 1 and 2B). Furthermore, Brennan allegedly inherently discloses a method for detecting different base compositions among nucleic acids of identical length by synthesizing the nucleic acids in the presence of nucleotide analogs (Abstract; Column 5, line 25 to Column 6, line 8; Examples 1-4; and Schemes D and E) that separate the masses of nucleic acids having different base compositions in a predetermined interval (Figures 1-2). Applicant respectfully traverses this rejection.

Relevant law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S.. 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention". In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984).

Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. Prior art does not anticipate a thing or process unless it is enabling; an anticipatory publication must describe the claimed invention with sufficient clarity and specificity so that one skilled in the relevant art could practice the subject matter of the patent without assistance from the patent claimed to have been anticipated Columbia Broadcasting System v. Sylvania Elec. Products, Inc., 415 F.2d 719, 735, 162 USPQ 577 (1st Cir.1968) cert. denied, 396 U.S. 1061, 164 USPQ 321 (1970).

"Before any publication can amount to a statutory bar to the grant of a patent, its disclosure must be such that a skilled artisan could take its teachings in combination with his own knowledge of the particular art and be in possession of the invention." Titanium Metals crp. v Mossinghoff, 603 F.Supp. 87,0, 225 USPQ 673 (1984) quoting In re Application of Le Grice 49 CCPA 1124, 301 F.2d 9333. An inherent property has to flow naturally from what is taught in a reference In re Oelrich, 666 F.2d 578; 212 USPQ 323 (CCPA 1981).

The Claims

Claim 1 is directed to a method for identifying a nucleotide at one or more base positions in a target nucleic acid molecule, including synthesizing extension products of the target nucleic acid in the presence of chain terminating nucleotides and mass-matched nucleotides; determining the mass of each extension product; and calculating a mass shift from a period for the mass of each extension product, whereby nucleotide(s) at one or more base positions is determined by identifying the nucleotide that corresponds to each mass shift. Claim 2 is directed to the method of claim 1 that is a method for determining a nucleotide sequence of a target nucleic acid by assigning a nucleotide corresponding to each mass shift. Claim 3 is directed to the method of claim 1 in which the mass-matched deoxynucleotides are identical.

Claim 5 is directed to a method for identifying nucleotides at one or more base positions in a plurality of target nucleic acids molecules, including synthesizing extension products of the target nucleic acid in the presence of chain terminating nucleotides and mass-matched nucleotides; determining the mass of each extension product; and calculating a mass shift from a period for the mass of each extension product, whereby the nucleotides in the target nucleic acid molecules are identified by determining the nucleotide that corresponds to each mass shift. Claim 6 is directed to the method of claim 5 that is a method for determining nucleotide sequences of a plurality of target nucleic acids molecules by determining the nucleotide that corresponds to each mass shift. Claim 7 is directed to the method of claim 5 in which the mass-matched deoxynucleotides are identical to one another.

Claim 10 is directed to a method for determining a nucleotide sequence of a target nucleic acid molecule, including incorporating pair-matched nucleotides into the target nucleic acid; synthesizing extension products of the target nucleic acid in the presence of a partially duplex hairpin primer, chain terminating nucleotides and pair-matched nucleotides; determining the mass of

each extension product; and calculating a mass shift from a period for the mass of each extension product; whereby the nucleotide sequence of the target nucleic acid is determined by assigning a nucleotide corresponding to each mass shift. Claim 11 is directed to the method of claim 10 in which the chain terminating nucleotides are mass-matched. Claim 12 is directed to the method of claim 10 in which the chain terminating nucleotide base pairs have distinct molecular weights.

Claim 25 is directed to a method for detecting different nucleotide base compositions in a population of nucleic acids having identical length, including synthesizing the nucleic acids in the presence of one or more nucleotide analogs to produce synthesized nucleic acids; and determining a mass of each synthesized nucleic acid; whereby different nucleotide base compositions are detected by determining the mass of each synthesized nucleic acid and the masses of nucleic acids having different base compositions are separated in a predetermined interval by the nucleotide analog(s). Claim 26 is directed to the method of claim 25 in which the population of nucleic acids having identical length and different base compositions differ in base composition by a single base.

Claim 27 is directed to a method for detecting a plurality of target nucleic acid molecules in a sample containing nucleic acid molecules, including preparing a composition containing plurality of pair-matched nucleic acid molecules or mass-matched nucleic acid molecules from a sample comprising the target nucleic acid molecules; analyzing the resulting composition by mass spectrometry; and detecting target nucleic acid molecules.

Thus, all of the claims have as elements either mass-matched or pair-matched nucleotides, generation of a period or calculation of a mass shift from such period (Claims 1, 5, 10, 27 and dependents), or separating nucleic acids of identical length according to base composition by incorporation of nucleotide analogs that separate the different base compositions in a pre-determined

interval (Claims 25 and 26).

Differences between the disclosure of Brennan and the claimed subject matter

Brennan is directed to an apparatus and reagents for sequencing a target nucleic acid. In Brennan, the target nucleic acid to be sequenced is subjected to Sanger sequencing reactions in which each chain-terminated fragment is associated with a base-specific nuclide that is detectable by mass spectrometry. The chain-terminated fragments are separated according to size by any separation method, such as gel electrophoresis, the size-separated fragments are sequentially introduced into a mass spectrometer, and the terminal base of each fragment is identified by mass spectrometric detection of a nuclide that is uniquely associated with the terminal base. The nuclides can be incorporated into the fragments during the A, G, C and T Sanger sequencing reactions by labeling the component primers, the dideoxynucleotides, or the deoxynucleotides with the nuclides, as long as each Sanger sequencing reaction (*i.e.*, A, G, C and T) is uniquely associated with a nuclide label.

The disclosure of Brennan bears no relevance to the instantly claimed subject matter. The instantly claimed methods are aimed, for example, at identifying nucleotides at one or more base positions in a target nucleic acid by measuring the masses of extension products of target nucleic acids with sufficient resolution and accuracy to unambiguously assign peak positions of the extension products according to their base position, base identity, and target sequence from which the fragments arose. In the instantly claimed methods, extension products of the target nucleic acid to be sequenced are synthesized using a primer and mass-matched or pair-matched nucleotides such that each extension product has a mass shift that deviates from a periodic relationship to the size of the original primer by an amount that is dependent on the base position and base identity of the terminal nucleotide. The periodicity of this relationship is generated by selecting the mass-matched or pair-matched nucleotides to maximize the mass separation between the fragments for optimal

resolution and accuracy. The claimed methods can also be tailored to separate nucleic acids of identical length but different base compositions by incorporating nucleotide analogs into the population of sequences of identical length such that the masses of nucleic acid sequences of identical length but different base compositions are separated by a predetermined interval that is dependent on the identity of the nucleotide analogs. The instantly claimed methods optimize the accuracy of measuring not only a single target nucleotide sequence or base composition, but a mixture of multiple sequences or base compositions, by optimizing resolution between the multiple sequences or base compositions.

Brennan does not disclose any method involving measuring the masses of extension products or of nucleic acid sequences, much less improving the resolution of separation between the masses of such fragments or sequences. There is no disclosure in Brennan of separation and analysis of fragments from even a single target sequence on a single spectrum, much less that of multiple target sequences. The method disclosed in Brennan merely detects previously separated fragments that are sequentially introduced into a mass spectrometer, by detection of a unique nuclide associated with each fragment according to its terminal base (A, G, C or T). Moreover, as discussed above, the mass spectrometric detection disclosed in Brennan does not measure the masses of extension products or of any nucleic acid sequences. The nuclides detected in Brennan include a set of sulfur nuclides ($^{32}S/^{33}S/^{34}S/^{36}S$) and a set of halogen nuclides ($^{35}Cl/^{37}Cl/^{79}Br/^{81}Br$), **and it is the masses of their combustion products, namely, SO_2, Cl_2 or Br_2, that are detected by mass spectrometry**.

Further, Brennan does not disclose using mass-matched nucleotides (nucleotides whose masses are identical) or pair-matched nucleotides (masses of base pairs being identical) to create a periodic distribution of extension products whose terminal base identities are determined by the mass shifts of the extension products from their corresponding periodic reference mass. **In fact, Brennan does not disclose mass-matched nucleotides or pair-matched**

nucleotides at all. There is no disclosure anywhere in Brennan in which nucleotide analogs generating a periodic distribution of extension products are all of identical mass, or form base pairs of identical mass. The instant specification defines these terms on page 15, line 29 through page 16, line 26 as follows:

"As used herein, the term "mass-matched nucleotides" refers to a set of nucleotide analogs wherein each analog is of **identical** mass to each of the other analogs." (emphasis added)

"As used herein, the term "pair-matched nucleotides" refers to a nucleotide set in which the nucleotide analogs are selected such that the total mass of each base pair is **identical**." (emphasis added)

Contrary to the Examiner's assertion, neither the Abstract, nor the disclosure from column 5, line 25 to column 6, line 8, of Brennan disclose using nucleotide analogs whose masses are identical to each other. Further, Examples 1-4 and Schemes D and E of Brennan disclose synthetic routes for incorporating sulfur and halogen nuclides into deoxynucleotides or dideoxynucleotides, the combustion products of which are detected by mass spectrometry and are used to identify the fragment (extension product) from which they are generated. Brennan does not disclose determining the masses of these extension products, and Brennan certainly does not disclose using mass-matched or pair-matched nucleotides to determine the masses of the extension products.

Furthermore, Brennan does not disclose the calculation of a mass shift from a period for the mass of each extension product. A mass shift is described in the instant specification as a deviation from the corresponding predetermined periodic reference mass, where the predetermined periodicity of distribution of the extension products is generated by incorporating or simulating the incorporation of mass-matched or pair-matched nucleotides into the extension products. As described, for example, in Examples 1 and 2 of the instant specification, the periodicity of distribution of the extension products or

sequences with respect to the reference primer is defined by the masses of either the mass-matched nucleotide (Example 1) or the pair-matched nucleotides (Example 2) that are incorporated by primer extension into the target nucleic acid sequences to be analyzed. **No such period is disclosed anywhere in Brennan**. The Abstract; column 5, line 25 to column 6, line 64; Examples 1-4, and Figure 2B of the specification in Brennan merely disclose chromatographic separation of nucleic acid fragments followed by their sequential mass spectrometric detection according to the masses of the combustion products generated by the nuclides incorporated into the fragments. Figure 2B of Brennan shows a plot of ion current *vs.* time for the m/e values of the combustion product (SO_2) produced by four isotopes of sulfur, ^{32}S, ^{33}S, ^{34}S and ^{36}S, each of which is associated with a unique base (A, G, C or T). Figure 2B shows that as each chromatographically separated nucleic acid fragment is sequentially introduced into the mass spectrometer, the mass spectrometer detects a mass of a nuclide combustion product, which mass is indicative of the identity of the base corresponding to the particular nuclide isotope. Accordingly, the sequence of bases can be read from the corresponding nuclide masses that are read from each sequentially introduced nucleic acid fragment. Brennan does not disclose measuring the masses of these fragments.

There is no disclosure whatsoever of incorporating nucleotide analogs that are mass-matched (masses of all nucleotides in the products generated from the target by primer extension are identical) or that are pair-matched (masses of all base pairs in the products generated from the target by primer extension are identical), much less selecting such analogs to generate a pre-determined periodicity of distribution of such products. Since there is no disclosure anywhere in Brennan of incorporating into extension products nucleotide analogs whose masses or the masses of whose base pairs are identical, **there is certainly no disclosure in Brennan where the nucleotide analogs are not only of identical mass, but are in fact one and the same**

nucleotide (*e.g.*, deoxyinosine, as disclosed in the instant specification). Moreover, since Brennan does not disclose any periodic distribution of extension products or other nucleic acid sequences that is equal to the masses of the mass-matched or pair-matched nucleotides, **Brennan cannot possibly disclose, and in fact does not disclose, a mass shift** that is a deviation from such periodic distribution in a manner that is unique to each base. As shown in, for example, Examples 1 and 2 of the instant specification, the mass shift determines the identity of a nucleotide at any base position in a target sequence, and is defined as the distance in daltons between the observed peak (mass of extension product) and the nearest periodic reference mass, which occurs with a periodicity (relative to the reference primer used to generate the extension products) that is equal to the mass of the mass-matched nucleotide or of the pair-matched nucleotides. No such mass shift is disclosed in Brennan.

Brennan merely detects size-separated fragments sequentially, by identifying, *via* mass spectrometry, the combustion products of nuclides that are uniquely associated with the individual bases. Brennan does not disclose modulating the distribution of these size-separated fragments in any way, much less by incorporating mass-matched or pair-matched nucleotides into the fragments by primer extension, and even less so by generating a periodic distribution of the fragments with respect to the size of the extension primer against which a mass shift in the mass of a fragment is measured to identify the sequence of the fragment.

Finally, contrary to the Examiner's assertions, Brennan does not explicitly or inherently disclose a method for detecting different nucleotide base compositions in a population of nucleic acids having identical length. As discussed earlier, contrary to the Examiner's assertions, Figures 1 and 2 (and column 11, lines 1-38) of Brennan merely disclose sequential mass spectrometric detection of the combustion products of nuclides associated with nucleic acid fragments that are already separated according to size (length), and

that are the products of sequential primer extension by Sanger sequencing modified to incorporate such nuclides. There is no disclosure anywhere in Brennan of separation or analysis of a mixture of nucleic acid fragments of identical length, much less doing so by introducing nucleic acid analogs into the fragments where the **analogs are selected to separate the fragments of identical length according to differences in base composition in a pre-determined interval**.

Because Brennan does not disclose mass-matched or pair-matched nucleotides, generation of a period or calculation of a mass shift from such period (Claims 1, 5, 10, 27 and dependents), nor does Brennan disclose a method for detecting different base compositions in a population of nucleic acids of identical length by incorporation of nucleotide analogs that separate the different base compositions in a pre-determined interval (Claims 25 and 26), Brennan does not anticipate the claims.

REJECTION OF CLAIMS 1-8, 10-20 and 25-45 UNDER 35 U.S.C. § 103(a)

Various subsets of claims 1-8, 10-20 and 25-45 are rejected under 35 U.S.C. §103(a) over Brennan in view of either Schulz (U.S. Patent No. 6,232,076 B1) (Claims 1-8, 10-12 and 25-27); Shuber (U.S. Patent No. 5,888,778) (Claims 1-3, 5-7, 10-12 and 25-45); or Canard *et al.* (U.S. Patent No. 5,798, 210) (Claims 1-3, 5-7, 10-20 and 25-27). It is alleged that it would have been *prima facie* obvious to one of skill in the art at the time the instant application was filed to combine Brennan, which allegedly teaches a method for identifying nucleotides at one or more base positions of target nucleic acid molecules by synthesizing extension products using either mass-matched or pair-matched nucleotides and chain terminating nucleotides, calculating a mass shift from a period for the mass of each extension product, and identifying the nucleotides at each base position that correspond to each mass shift, with either Schulz, which allegedly teaches a method in which the mass-matched deoxynucleotide is deoxyinosine; Shuber, which allegedly teaches a method for detecting a mutation in a target nucleic acid sequence; or Canard *et al.*, which

allegedly teaches a method where the primers are duplex hairpins, to arrive at the instantly claimed subject matter. These rejections are respectfully traversed.

Relevant law

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. § 103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (*ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed subject matter. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed subject matter, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v Montefiore Hosp.* 732 F.2d 1572, 1577. 221 USPQ 929, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. *Stratoflex Inc. v Aeroquip Corp.*, 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). In addition, the mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23

USPQ 1783 (Fed. Cir. 1992).

Also, it is impermissible to ignore the advantages, properties, utilities and unexpected results that flow from the claimed invention; they are part of the invention as a whole. *In re Sernaker*, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983). Unexpected properties must always be considered when determining obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. *In re Papesh*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

A. REJECTION OF CLAIMS 1-8, 10-12 AND 25-27 UNDER 35 U.S.C. § 103(a) OVER BRENNAN IN VIEW OF SCHULZ

Claims 1-8, 10-12, and 25-27 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Brennan in view of Schulz (U.S. Patent No. 6,232,076). It is alleged that it would have been *prima facie* obvious to one of skill in the art at the time the instant application was filed to combine Brennan, which, as set forth by the Examiner with respect to the rejection on grounds of anticipation, allegedly teaches a method for identifying nucleotides at one or more base positions of target nucleic acid molecules by synthesizing extension products using either mass-matched or pair-matched nucleotides and chain terminating nucleotides, calculating a mass shift from a period for the mass of each extension product, and identifying the nucleotides at each base position that correspond to each mass shift, with Schulz, which allegedly teaches a method in which the mass-matched deoxynucleotide is deoxyinosine, to arrive at the instantly claimed subject matter. This rejection is respectfully traversed.

The Claims

Claims 1-3, 5-7, 10-12 and 25-27 are as described above. Claim 4 is directed to the method of Claim 1 and Claim 8 is the method of Claim 5 where the mass-matched deoxynucleotide is deoxyinosine, 5-nitroindole, 3-nitropyrrole, 3-methyl 7-propynyl isocarbostyril, 5-methyl iscarbostyril or 3-methyl isocarbostyril.

Thus, all of the claims are directed to methods involving either mass-matched or pair-matched nucleotides, generation of a period and calculation of a mass shift from such period, or separating nucleic acids of identical length according to base composition by incorporation of nucleotide analogs that separate the different base compositions in a pre-determined interval.

Teachings of the cited art, and the differences between the teachings of the cited art and claims 1-8, 10-12 and 25-27

Brennan

As described above, Brennan teaches the mass spectrometric detection of chain-terminated fragments of target nucleic acid sequences by incorporating into the fragments, *via* Sanger sequencing reactions, base-specific nuclides whose combustion products are detectable by mass spectrometry. Brennan teaches the association of each nuclide with a unique base, and Brennan further teaches that the chain terminal base in each fragment may be identified by detection of the nuclide with which the base is uniquely associated. For example, Brennan teaches that a set of four nuclides, each of which is uniquely associated with a base, may be a set of sulfur nuclides ($^{32}S/^{33}S/^{34}S/^{36}S$) or a set of halogen nuclides ($^{35}Cl/^{37}Cl/^{79}Br/^{81}Br$). Brennan teaches that the nuclides are detected by their combustion products (*e.g.*, SO_2, Cl_2 or Br_2), and the masses of the combustion products (and, therefore, the unique base with which each nuclide is associated) are distinguished by the difference in the masses of the nuclide isotopes.

Brennan does not teach or suggest identifying nucleotides at one or more positions in a target nucleic acid sequence by measuring the masses of the extension products synthesized from the target sequence. In Brennan, the terminal nucleotide of each extension fragment is identified by the mass of the combustion product of a nuclide that is associated with the terminal nucleotide.

Further, there is absolutely no teaching or suggestion in Brennan of using mass-matched nucleotides (nucleotides whose masses are identical) or pair-matched nucleotides (masses of base pairs being identical) to synthesize

extension products so as to create a periodic distribution of the extension products whose terminal base identities are determined by their mass shift from their corresponding periodic reference mass. Nowhere in Brennan is there any teaching or suggestion of incorporating nucleotide analogs into the extension products where the masses of all the nucleotide analogs used to synthesize the extension products are identical to each other (mass-matched nucleotides), or masses of base pairs formed by the nucleotide analogs are identical (pair-matched nucleotides).

Furthermore, Brennan does not teach or suggest the calculation of a mass shift from a period for the mass of each extension product. A mass shift is described in the instant specification as a deviation from the corresponding predetermined periodic reference mass, where the predetermined periodicity of distribution of the extension products is generated by incorporating mass-matched or pair-matched nucleotides into the extension products. As described, for example, in Examples 1 and 2 of the instant specification, the periodicity of distribution of the extension products or sequences with respect to the reference primer is defined by the masses of either the mass-matched nucleotide (Example 1) or the pair-matched nucleotides (Example 2) that are incorporated by primer extension into the target nucleic acid sequences to be analyzed. There is absolutely no teaching or suggestion anywhere in Brennan of generating a periodic distribution of the extension products. Brennan merely teaches sequential mass spectrometric identification of terminal nucleotides in extension products that have already been separated by other means, such as gel electrophoresis. There is no teaching of any modulation of the separation between extension products, much less that of any periodic separation as described in the instant application.

Since Brennan does not teach or suggest incorporating into extension products nucleotide analogs whose masses or the masses of whose base pairs are identical, there is certainly no teaching in Brennan that the nucleotide

analogs are not only of identical mass, but are in fact one and the same nucleotide (*e.g.*, deoxyinosine, as described in the instant specification). Moreover, since Brennan does not teach or suggest any periodic distribution of extension products or other nucleic acid sequences that is equal to the masses of the mass-matched or pair-matched nucleotides, Brennan certainly cannot teach or suggest calculating a mass shift that is a deviation from such periodic distribution in a manner that is unique to each base. Brennan merely teaches the detection of size-separated fragments sequentially, by identifying, *via* mass spectrometry, the combustion products of nuclides that are uniquely associated with the individual bases. Brennan does not teach or suggest modulating the distribution of these size-separated fragments in any way, much less by incorporating mass-matched or pair-matched nucleotides into the fragments by primer extension, and even less so by generating a periodic distribution of the fragments with respect to the size of the extension primer against which a mass shift in the mass of a fragment is measured to identify the sequence of the fragment.

Brennan also does not teach or suggest a method for detecting different nucleotide base compositions in a population of nucleic acids having identical length. As discussed earlier, contrary to the Examiner's assertions, Figures 1 and 2 (and column 11, lines 1-38) of Brennan merely teach sequential mass spectrometric detection of the combustion products of nuclides associated with nucleic acid fragments that are already separated according to size (length), and that are the products of sequential primer extension by Sanger sequencing modified to incorporate such nuclides. There is no teaching or suggestion anywhere in Brennan of separation or analysis of a mixture of nucleic acid fragments of identical length, much less doing so by introducing nucleic acid analogs into the fragments where the analogs are selected to separate the fragments of identical length according to differences in base composition in a pre-determined interval.

Schulz

Schulz teaches a method of reducing degradation of polymerase chain extension products containing a nonradioactive detection moiety by adding a base, buffer or reducing agent. Schulz also teaches methods that generate extension products containing nonradioactive detection moieties, such as fluorescently labeled nucleotides, and Schulz teaches that nucleotides that may be used in these methods include deoxyinosine. Schulz does not teach or suggest that deoxyinosine may be used as a mass-matched nucleotide.

Schulz does not teach or suggest synthesizing extension products in the presence of mass-matched or pair-matched nucleotides. As discussed earlier, the extension reactions described in the instant application incorporate nucleotide analogs that are all of identical mass (mass-matched), or form base pairs of identical mass (pair-matched). Nowhere does Schulz teach or suggest using mass-matched or pair-matched nucleotides in polymerase extension reactions, nor that mass-matched or pair-matched nucleotides can be used to generate a periodic distribution of the masses of the extension products, nor that a mass shift that is a deviation from the periodic distribution is used to identify nucleotides at one or more positions in a target nucleic acid sequence. There is also no teaching or suggestion anywhere in Schulz of separation or analysis of a mixture of nucleic acid fragments of identical length, much less doing so by introducing nucleic acid analogs into the fragments where the analogs are selected to separate the fragments of identical length according to differences in base composition in a pre-determined interval.

The Examiner has failed to set forth a *prima facie* case of obviousness.

Analysis

The combination of teachings of the cited references does not result in the instantly claimed methods

It is alleged that it would have been *prima facie* obvious to one of skill in the art at the time the instant application was filed to combine Brennan, which, as set forth by the Examiner with respect to the rejection on grounds of

anticipation, allegedly teaches a method for identifying nucleotides at one or more base positions of target nucleic acid molecules by synthesizing extension products using either mass-matched or pair-matched nucleotides and chain terminating nucleotides, calculating a mass shift from a period for the mass of each extension product, and identifying the nucleotides at each base position that correspond to each mass shift, with Schulz, which allegedly teaches a method in which the mass-matched deoxynucleotide is deoxyinosine.

As noted above, neither of the cited references teaches or suggests any of the elements of measuring the masses of extension products, incorporating mass-matched or pair-matched nucleotides into extension products, generating a periodic distribution of the masses of the extension products or determining a mass shift that identifies nucleotides at one or more positions in a target sequence. Therefore, the combination of the cited references does not lead to the instantly claimed subject matter.

The Office Action provides that Brennan allegedly teaches the use of mass-matched and pair-matched nucleotides for determining sequence data, but does not teach the use of deoxyinosine as a mass-matched nucleotide, which use is allegedly taught by Schulz. However, as described above, nowhere does Brennan teach or suggest a method for identifying nucleotides in a target sequence by synthesizing extension products in the presence of mass-matched or pair-matched nucleotides. As defined in the instant specification and as discussed above, mass-matched nucleotides are a set of nucleotides all having the same mass, and pair-matched nucleotides are a set of nucleotides whose base pairs are of identical mass. Brennan teaches a method for identifying nucleotides in chromatographically separated extension fragments by mass spectrometric detection of the combustion products of nuclides (sulfur or halogen, *e.g.*, producing sulfur dioxide or halogen gases that are detectable by mass spectrometry) associated with the nucleotides. Nowhere does Brennan teach or suggest using a set of nucleotides having identical masses, or the

masses of whose base pairs are identical.

Schulz, which teaches primer extension reactions to generate extension products containing nonradioactive detection moieties, does not cure these deficiencies. The Office Action alleges that Schulz teaches the use of deoxyinosine as a mass-matched deoxynucleotide. As discussed earlier, the instant specification describes the synthesis of extension products using nucleotide analogs of identical mass (mass-matched) or whose base pairs are of identical mass (pair-matched) where the masses of the extension products follow a periodic distribution that is equal to the mass of the mass-matched or pair-matched nucleotides. While Schulz teaches deoxyinosine as a nucleotide that may be used in primer extension reactions that incorporate nonradioactive detection moieties, Schulz contains no teaching or suggestion whatsoever of generating extension products containing mass-matched or pair-matched nucleotides. In fact, Schulz does not mention mass-matched or pair-matched nucleotides at all. Therefore, there is certainly no teaching or suggestion in Schulz that deoxyinosine is a mass-matched nucleotide.

Additionally, neither Brennan nor Schulz teaches or suggests generating a periodic distribution of the masses of extension products, nor of calculation of a mass shift based on a deviation of the mass of an extension product from its corresponding periodic reference mass such that the value of the mass shift identifies both the type and the position of one or more nucleotides in a target nucleic acid molecule. Therefore, the combination of these cited references does not lead to the use of deoxyinosine in primer extension reactions such that deoxyinosine is a mass-matched nucleotide that generates a periodic distribution of extension products whose masses deviate from the corresponding reference mass by a value that is equal to the mass shift.

As discussed above, the instantly claimed methods are directed to the identification of nucleotides at one or more positions in a target nucleic acid molecule by synthesizing extension products of the target nucleic acid using a

primer and mass-matched or pair-matched nucleotides such that each extension product has a mass shift that deviates from a periodic relationship to the size of the original primer by an amount that is dependent on the base position and base identity of the terminal nucleotide. The claimed methods can also be tailored to separate nucleic acids of identical length but different base compositions by incorporating nucleotide analogs into the population of sequences of identical length such that the masses of nucleic acid sequences of identical length but different base compositions are separated by a predetermined interval that is dependent on the identity of the nucleotide analogs.

Neither of the cited references, singly or in combination, teaches or suggests the elements of the instant claims. The cited references, singly or in combination, fail to teach or suggest mass-matched or pair-matched nucleotides, a periodic distribution of extension products that are synthesized using mass-matched or pair-matched nucleotides, calculation of a mass shift of each extension product from a periodic reference mass, or separation of a population of nucleic acid molecules of identical length according to their base composition by incorporating nucleotide analogs into the population such that the separation according to base composition occurs in a pre-determined interval.

Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

B. REJECTION OF CLAIMS 1-3, 5-7, 10-12 AND 25-45 UNDER 35 U.S.C. § 103(a) OVER BRENNAN IN VIEW OF SHUBER

Claims 1-3, 5-7, 10-12 and 25-45 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Brennan in view of Shuber (U.S. Patent No. 5,888,778). It is alleged that it would have been *prima facie* obvious to one of skill in the art at the time the instant application was filed to combine Brennan, which allegedly teaches a method for identifying nucleotides at one or more base positions of target nucleic acid molecules by synthesizing

extension products using either mass-matched or pair-matched nucleotides and chain terminating nucleotides, calculating a mass shift from a period for the mass of each extension product, and identifying the nucleotides at each base position that correspond to each mass shift, with Shuber, which allegedly teaches a method for detecting a mutation in a target nucleic acid sequence in which primers are hybridized to the target sequence at a region adjacent to a suspected mutation, and the primers are extended in the presence of a composition containing mass-matched nucleotides and chain terminating nucleotides, to arrive at the instantly claimed subject matter. This rejection is respectfully traversed.

The Claims

Claims 1-3, 5-7, 10-12 and 25-27 are described above.

Claim 28 is directed to a process for detecting a mutation in a target sequence in a target nucleic acid molecule involving hybridizing a primer that is complementary to a sequence in the target nucleic acid that is adjacent to the region suspected of containing a mutation and where the target nucleic acid may or may not be immobilized; contacting the hybridized primer with a composition containing mass-matched deoxyribonucleoside triphosphates and a chain terminating nucleotide selected from a dideoxyribonucleoside triphosphate or a 3'-deoxynucleoside triphosphate and optionally one or more deoxyribonucleoside triphosphates, such that the hybridized primer is extended until a chain terminating nucleotide is incorporated; and determining the mass of the extended primer, thereby determining whether a mutation is present in the target nucleic acid sequence. Claim 29 specifies that the chain terminating nucleotides of claim 28 are mass-matched, and Claim 30 specifies that the mass of the extended primer of claim 28 is determined by mass spectrometry.

Claim 31 is directed to a process that follows the steps of Claim 28, except that a plurality of target nucleic acid sequences are analyzed. Claim 32 specifies that the chain terminating nucleotides of claim 28 are mass-matched,

and Claim 33 specifies that the mass of the extended primer of claim 28 is determined by mass spectrometry

Claim 34 is directed to a method for detecting a target nucleic acid sequence in which a primer hybridized to a nucleic acid molecule containing a target nucleic acid sequence can be extended in a 3′ direction towards the target nucleic acid sequence; the 5′ end of the primer can be selectively cleaved from the extension product; the primer is extended in the presence of mass matched deoxyribonucleotides and a polymerase to produce an extension product; the 5′ end of the primer can be cleaved from the extension product to produce a portion of the primer and a cleaved extension product; and the cleaved extension product is detected. Claim 35 is directed to the method of claim 34 where the cleaved extension product is detected by mass spectrometry.

Claim 36 is directed to a method that follows the steps of Claim 34, except that a plurality of target nucleic acid sequences are simultaneously analyzed. Claim 37 is directed to the method of claim 36 where the cleaved extension product is detected by mass spectrometry.

Claim 38 is directed to a method for detecting a target nucleic acid sequence, including hybridizing to a nucleic acid molecule containing the target nucleic acid sequence a first primer, which can be extended in a 3′ direction towards the target nucleic acid sequence and where the 5′ end of the primer can be selectively cleaved from the extension product, and a second primer, which can be extended in a 3′ direction towards the first primer; extending the primers in the presence of mass-matched nucleotides to produce a double stranded amplification product; selectively cleaving the 5′ end of the first primer in the amplification product, to produce a double stranded amplification product containing a cleaved primer extension product with a 5′ portion and a 3′ portion; denaturing the extension product; and detecting the 3′ portion of the cleaved primer extension product. Claim 39 is directed to the method of claim

38 where the cleaved extension product is detected by mass spectrometry.

Claim 40 is directed to a method that follows the steps of Claim 38, except that a plurality of target nucleic acid sequences are simultaneously analyzed. Claim 41 is directed to the method of claim 40 where the cleaved extension product is detected by mass spectrometry.

Claim 42 is directed to a method for detecting a target nucleic acid sequence, including hybridizing first and second primers to a nucleic acid molecule containing the target nucleic acid sequence, where a primer contains a selectively cleavable site at its 3' end; extending the primers in the presence of mass-matched nucleotides; cleaving the resulting product at the selectively cleavable sites; and analyzing the masses of the cleavage products, whereby the target sequence is detected. Claim 43 specifies that the cleaved extension product of claim 42 is detected by mass spectrometry. Claim 44 is directed to the method of claim 43 where a plurality of primers are hybridized and a plurality of target sequences are identified in a single reaction. Claim 45 is directed to the method of claim 44, where the cleaved extension products are detected by mass spectrometry.

Thus, all of the claims are directed to methods involving either mass-matched or pair-matched nucleotides, generation of a period and calculation of a mass shift from such period, separating nucleic acids of identical length according to base composition by incorporation of nucleotide analogs that separate the different base compositions in a pre-determined interval, or primer extension for detecting mutations in a target nucleic acid or for sequencing a target nucleic acid by analysis of cleaved primer extension products that contain mass-matched nucleotides incorporated during the primer extension.

Teachings of the cited art, and the differences between the teachings of the cited art and claims 1-3, 5-7, 10-12 and 25-45

The teachings of Brennan are discussed above.

Shuber

Shuber is directed to methods for detecting and identifying genetic

mutations or the presence of disease-causing microorganisms in a biological sample. In Shuber, single-base extensions are carried out using segmented primers that bind to the template sequence in a cooperative fashion that eliminates false priming. Shuber teaches that one of the segmented primers is immediately upstream of a genetic alteration, and that extension of this primer detects and identifies genetic alterations or part of a foreign genetic sequence such as the genome of an invading disease-causing organism.

Shuber does not teach or suggest synthesizing extension products in the presence of mass-matched or pair-matched nucleotides, nor of measuring the masses of extension products. Further, nowhere does Shuber teach or suggest using mass-matched or pair-matched nucleotides in polymerase extension reactions, nor that mass-matched or pair-matched nucleotides can be used to generate a periodic distribution of the masses of the extension products, nor that a mass shift that is a deviation from the periodic distribution is used to identify nucleotides at one or more positions in a target nucleic acid sequence. The instant application teaches that in a primer extension reaction using mass-matched or pair-matched nucleotides, incorporation of a chain terminator will indicate the presence of a mutation or provide information about the target sequence by a shift in periodicity from a periodic reference mass whose is value is equal to that of a mass-matched or pair-matched nucleotide. Not only does Shuber not teach or suggest mass-matched nucleotides, there is no teaching or suggestion in Shuber that mutations or other sequence alterations can be detected by the masses of the extension products formed by primer extension, that the primers have selectively cleavable sites, or that the mutation would be detected because of a deviation in periodicity of the masses of the cleaved extension products.

There is also no teaching or suggestion anywhere in Shuber of separation or analysis of a mixture of nucleic acid fragments of identical length, much less doing so by introducing nucleic acid analogs into the fragments where the

analogs are selected to separate the fragments of identical length according to differences in base composition in a pre-determined interval.

The Examiner has failed to set forth a *prima facie* case of obviousness.

Analysis

The combination of teachings of the cited references does not result in the instantly claimed methods

Neither of the cited references, singly or in combination, teaches or suggests measuring the masses of extension products, incorporating mass-matched or pair-matched nucleotides into extension products, generating a periodic distribution of the masses of the extension products or determining a mass shift that identifies nucleotides at one or more positions in a target sequence. Further, neither of the references, singly or in combination, teaches or suggests separating nucleic acids of identical length according to base composition by incorporation of nucleotide analogs that separate the different base compositions in a pre-determined interval. Furthermore, neither of the references, singly or in combination, teaches or suggests primers having selectively cleavable sites, nor of primer extension for detecting mutations in a target nucleic acid or for sequencing a target nucleic acid by analysis of cleaved primer extension products incorporating mass-matched nucleotides. Therefore, the combination of the cited references does not lead to the instantly claimed subject matter.

It is alleged in the Office Action that it would have been *prima facie* obvious to combine the method of Brennan, which allegedly teaches a method involving the use of mass-matched and pair-matched nucleotides and measuring the masses of the extension products for determining sequence data, with the method of Shuber, which allegedly teaches the use of single and multiple primers to detect mutations with high target selectivity, to arrive at the instantly claimed subject matter. However, as discussed above, nowhere does Brennan teach or suggest a method for identifying nucleotides in a target sequence by synthesizing extension products in the presence of mass-matched or pair-

matched nucleotides, nor of measuring the masses of the extension products. As discussed above, Brennan teaches a method for identifying nucleotides in chromatographically separated extension fragments by mass spectrometric detection of the combustion products of nuclides (sulfur or halogen, *e.g.*, producing sulfur dioxide or halogen gases that are detectable by mass spectrometry) associated with the nucleotides. Nowhere does Brennan teach or suggest using a set of nucleotides having identical masses (mass-matched), or the masses of whose base pairs are identical (pair-matched).

Shuber, which teaches the use of segmented primers for primer binding and extension adjacent to a genetically altered sequence or mutation with high selectivity, does not cure these deficiencies. The Examiner alleges that Shuber teaches using mass-matched nucleotides to produce primer extension products. The Examiner cites to Shuber at column 7, lines 38-59, and claims 1, 10, and 13, for support. However, the cited portions of Shuber merely teach the detection of mutations by primer extension using segmented primers, deoxynucleotides, and chain-terminating nucleotides. Nowhere does Shuber teach or suggest carrying out primer extension in the presence of mass-matched nucleotides, using primers having selectively cleavable sites, cleaving the extended product and measuring the mass of the cleavage product. As discussed earlier, the instant specification describes the synthesis of primer extension products using nucleotide analogs of identical mass (mass-matched, *i.e.*, the masses of all the deoxynucleotide analogs used in the extension reaction are identical) or whose base pairs are of identical mass (pair-matched), where the masses of the extension products follow a periodic distribution that is equal to the mass of the mass-matched or pair-matched nucleotides. The instant specification teaches that a deviation or mass shift from the periodic distribution, calculated by subtracting the mass of the extension product from the corresponding periodic reference mass, is indicative of the nucleotide sequence at a particular position in the target nucleic acid molecule, which

sequence may be informative of the presence of a mutation. While Shuber teaches the detection of mutations by primer extension using segmented primers, in which deoxyinosine may be used as a deoxynucleotide analog, nowhere is there any teaching or suggestion in Shuber of using deoxyinosine or any other deoxynucleotide analog as a mass-matched nucleotide and analyzing the masses of extension products that incorporate mass-matched nucleotides.

Additionally, neither Brennan nor Shuber teaches or suggests generating a periodic distribution of the masses of extension products, nor of calculation of a mass shift based on a deviation of the mass of an extension product from its corresponding periodic reference mass such that the value of the mass shift identifies both the type and the position of one or more nucleotides in a target nucleic acid molecule. In fact, there is no teaching or suggestion whatsoever in any of the cited references, nor in any combination thereof, of carrying out primer extension reactions in the presence of deoxynucleotides of identical mass or whose base pairs are of identical mass.

As discussed above, the instantly claimed methods are directed to the identification of nucleotides at one or more positions in a target nucleic acid molecule by synthesizing extension products of the target nucleic acid using a primer and mass-matched or pair-matched nucleotides such that each extension product has a mass shift that deviates from a periodic relationship to the size of the original primer by an amount that is dependent on the base position and base identity of the terminal nucleotide. The claimed methods can also be tailored to separate nucleic acids of identical length but different base compositions by incorporating nucleotide analogs into the population of sequences of identical length such that the masses of nucleic acid sequences of identical length but different base compositions are separated by a predetermined interval that is dependent on the identity of the nucleotide analogs. Furthermore, the claimed methods are directed to primer extension using primers having selectively cleavable sites for detecting mutations in a

target nucleic acid or for sequencing a target nucleic acid by analysis of cleaved primer extension products that contain mass-matched nucleotides incorporated during the primer extension.

Neither of the cited references, singly or in combination, teaches or suggests the elements of the instant claims. The cited references, singly or in combination, fail to teach or suggest mass-matched or pair-matched nucleotides, a periodic distribution of extension products that are synthesized using mass-matched or pair-matched nucleotides, calculation of a mass shift of each extension product from a periodic reference mass, separation of a population of nucleic acid molecules of identical length according to their base composition by incorporating nucleotide analogs into the population such that the separation according to base composition occurs in a pre-determined interval, or primer extension for detecting mutations in a target nucleic acid or for sequencing a target nucleic acid by analysis of cleaved primer extension products that contain mass-matched nucleotides incorporated during the primer extension.

Because neither reference cures the deficiencies in the other to arrive at the claimed subject matter, and because neither reference alone teaches or suggests the instantly claimed subject matter, the cited references, singly or in combination, fails to result in the instantly claimed subjected matter. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

C. REJECTION OF CLAIMS 1-3, 5-7, 10-20 AND 25-27 UNDER 35 U.S.C. § 103(a) OVER BRENNAN IN VIEW OF CANARD *ET AL.*

Claims 1-3, 5-7, 10-20 and 25-27 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Brennan in view of Canard (U.S. Patent No. 5,888,778). It is alleged that it would have been *prima facie* obvious to one of skill in the art at the time the instant application was filed to combine Brennan, which allegedly teaches a method for identifying nucleotides at one or more base positions of target nucleic acid molecules by synthesizing extension products using either mass-matched or pair-matched nucleotides and

chain terminating nucleotides, calculating a mass shift from a period for the mass of each extension product, and identifying the nucleotides at each base position that correspond to each mass shift, with Canard *et al.*, which allegedly teaches a method where the primers are duplex hairpins, to arrive at the instantly claimed subject matter. This rejection is respectfully traversed.

The Claims

Claims 1-3, 5-7, 10-12 and 25-27 are described above.

Claim 13 is directed to a method for determining nucleotide sequences of a plurality of target nucleic acids that includes incorporating pair-matched nucleotides into the target nucleic acids; synthesizing extension products of the target nucleic acids in the presence of a partially duplex hairpin primer, chain terminating nucleotides and pair-matched nucleotides; amplifying the target nucleic acid sequences in the presence of pair-matched nucleotides; determining the mass of each extension product; and calculating a mass shift from a period for the mass of each extension product; whereby the nucleotide sequences of the target nucleic acids are determined by assigning a nucleotide corresponding to each mass shift. Claim 14 is directed to the method of claim 13 where the chain terminating nucleotides are mass-matched. Claim 15 is directed to the method of claim 13 where the chain terminating nucleotide base pairs have distinct molecular weights. Claim 16 is directed to the method of claim 13 where the primers are mass-labeled.

Claim 17 is directed to a method for detecting one or a plurality of target nucleic acid(s) or one or plurality of nucleotides therein, which includes the steps of copying the target nucleic acid molecule(s) in the presence of a pair-matched set of nucleotides; denaturing the resulting copies of the target(s) to produce single-stranded templates; annealing and ligating one or a plurality of partially duplex hairpin primers to the single-stranded template(s); extending the primer(s) in the presence of chain terminating nucleotides and pair-matched nucleotides to produce extension products, where the extension products follow

a periodic mass distribution that is determined by the mass of the pair-matched nucleotide set; and detecting each of the targets or nucleotides therein by virtue of the mass shift of each extension product from its corresponding periodic reference mass. Claim 18 is directed to the method of claim 17 where the chain terminating nucleotides are mass-matched. Claim 19 is directed to the method of claim 17 where the chain terminating nucleotide base pairs have distinct molecular weights. Claim 20 is directed to the method of claim 17 where the primers are mass-labeled.

Thus, all of the claims are directed to methods involving either mass-matched or pair-matched nucleotides, generation of a period and calculation of a mass shift from such period, separating nucleic acids of identical length according to base composition by incorporation of nucleotide analogs that separate the different base compositions in a pre-determined interval and sequencing or detecting a plurality of target nucleic acids by determining a mass shift for extension products synthesized by extending duplex hairpin primers annealed to the target nucleic acids in the presence of chain terminating nucleotides and pair-matched nucleotides.

Teachings of the cited art, and the differences between the teachings of the cited art and claims 1-3, 5-7, 10-20 and 25-27

The teachings of Brennan are discussed above.

Canard *et al.*

Canard *et al.* is directed to the synthesis of 3'-hydroxy esters of deoxynucleotides that are characterized by unique fluorescent labels, one for each nucleotide. Canard *et al.* also teaches a method of sequencing a target nucleic acid molecule in which incorporation of a 3'-hydroxy ester derivative of a nucleotide during extension of a primer annealed to the target template results in chain termination. Canard *et al.* further teaches that this chain termination reaction is reversible because under certain conditions, *e.g.*, basic conditions, the ester derivative can be hydrolyzed to release the fluorescent label with

excitation and emission wavelengths that are unique for a given base. Furthermore, Canard *et al.* teaches that after hydrolysis to release the fluorescent label, the chain extension can be continued on the same template target nucleic acid molecule to identify the next nucleotide in the sequence. Thus, Canard *et al.* teaches a modification of the Sanger sequencing method in which the same sequencing reaction, rather than a set of four sequencing reactions (one for each nucleotide), can be used to sequentially identify nucleotides in a target nucleic acid molecule.

The teachings of Canard *et al.* are completely irrelevant to those of the instantly claimed subject matter. In the sequencing method discussed above, Canard *et al.* teaches that the use of hairpin primers for the extension reactions makes it possible to use "basic conditions for hydrolysis of the 3'-hydroxyl esters that is compatible with a repetition of the [chain extension] procedure without addition of a primer at each step of the indirect determination of a nucleotide inserted." (column 4, line 67 to column 5, line 3). The adaptability of the hairpin primer to the sequencing method of Canard *et al.* has no bearing on the instantly claimed subject matter, which does not involve hydrolysis under basic or any other conditions of chain terminal 3'-hydroxy protecting (ester) groups followed by continued chain extension to detect the next nucleotide in the sequence. Canard *et al.* does not teach or suggest extending duplex hairpin primers annealed to the target nucleic acids in the presence of chain terminating nucleotides and pair-matched nucleotides for the detection or sequencing of a plurality of target nucleic acids.

Further, Canard *et al.* does not teach or suggest synthesizing extension products in the presence of mass-matched or pair-matched nucleotides, nor of measuring the masses of extension products. Furthermore, nowhere does Canard *et al.* teach or suggest using mass-matched or pair-matched nucleotides in polymerase extension reactions, nor that mass-matched or pair-matched nucleotides can be used to generate a periodic distribution of the masses of the

extension products, nor that a mass shift that is a deviation from the periodic distribution is used to identify nucleotides at one or more positions in a target nucleic acid sequence.

There is also no teaching or suggestion anywhere in Canard *et al.* of separation or analysis of a mixture of nucleic acid fragments of identical length, much less doing so by introducing nucleic acid analogs into the fragments where the analogs are selected to separate the fragments of identical length according to differences in base composition in a pre-determined interval.

The Examiner has failed to set forth a *prima facie* case of obviousness.

Analysis

The combination of teachings of the cited references does not result in the instantly claimed methods

Neither of the cited references, singly or in combination, teaches or suggests measuring the masses of extension products, incorporating mass-matched or pair-matched nucleotides into extension products, generating a periodic distribution of the masses of the extension products or determining a mass shift that identifies nucleotides at one or more positions in a target sequence. Further, neither of the references, singly or in combination, teaches or suggests separating nucleic acids of identical length according to base composition by incorporation of nucleotide analogs that separate the different base compositions in a pre-determined interval. Furthermore, neither of the references, singly or in combination, teaches or suggests sequencing or detecting a plurality of target nucleic acids by determining a mass shift for extension products synthesized by extending duplex hairpin primers annealed to the target nucleic acids in the presence of chain terminating nucleotides and pair-matched nucleotides. Therefore, the combination of the cited references does not lead to the instantly claimed subject matter.

It is alleged in the Office Action that it would have been *prima facie* obvious to one of skill in the art to substitute and combine the method of Brennan with the method of Canard *et al.* because the use of hairpin primers

makes it possible to use basic conditions for deprotection of the 3'-hydroxyl that is compatible with a repetition of primer extension in a sequencing reaction without addition of primer at each step of the indirect determination of a nucleotide inserted. However, as discussed above, the adaptability of the hairpin primer to the sequencing method of Canard *et al.* has no bearing on the instantly claimed subject matter, which does not involve deprotection/hydrolysis under basic or any other conditions of chain terminal 3'-hydroxy protecting (ester) groups, followed by continued chain extension to detect the next nucleotide in the sequence. Neither Canard *et al.* nor Brennan nor the combination thereof teaches or suggests extending duplex hairpin primers annealed to the target nucleic acids in the presence of chain terminating nucleotides and pair-matched nucleotides for the detection or sequencing of a plurality of target nucleic acids.

As discussed above, Brennan teaches a method for identifying nucleotides in chromatographically separated extension fragments by mass spectrometric detection of the combustion products of nuclides (sulfur or halogen, *e.g.*, producing sulfur dioxide or halogen gases that are detectable by mass spectrometry) associated with the nucleotides. Nowhere does Brennan teach or suggest using a set of nucleotides having identical masses (mass-matched), or the masses of whose base pairs are identical (pair-matched). Canard *et al.*, which teaches a method of sequencing a target nucleic acid by incorporating a 3'-hydroxy ester derivative of a nucleotide during extension of a primer annealed to the target template, does not cure these deficiencies.

There is no teaching or suggestion whatsoever in any of the cited references, nor in any combination thereof, of carrying out primer extension reactions in the presence of deoxynucleotides of identical mass or whose base pairs are of identical mass. The cited references, singly or in combination, fail to teach or suggest mass-matched or pair-matched nucleotides, a periodic distribution of extension products that are synthesized using mass-matched or

pair-matched nucleotides, calculation of a mass shift of each extension product from a periodic reference mass, separation of a population of nucleic acid molecules of identical length according to their base composition by incorporating nucleotide analogs into the population such that the separation according to base composition occurs in a pre-determined interval, or sequencing or detecting a plurality of target nucleic acids by determining a mass shift for extension products synthesized by extending duplex hairpin primers annealed to the target nucleic acids in the presence of chain terminating nucleotides and pair-matched nucleotides. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

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In view of the above, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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